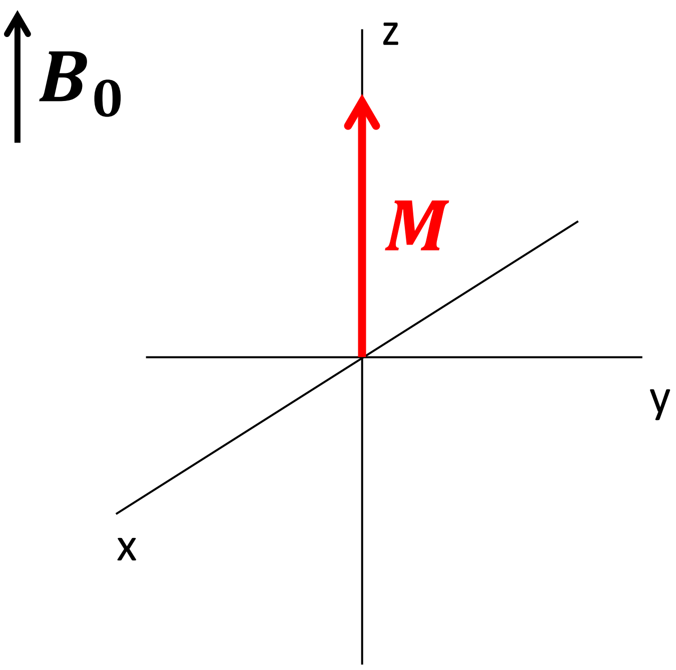
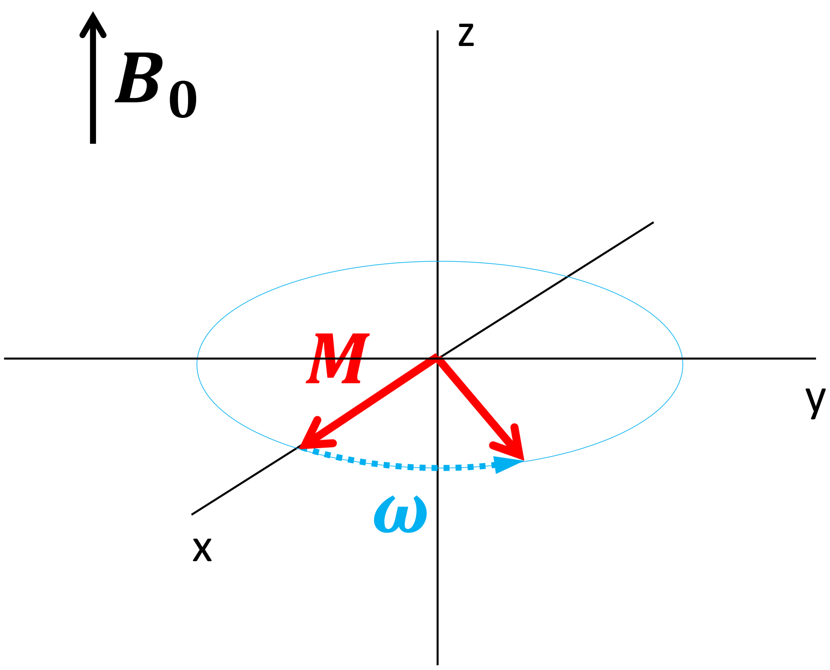
Magnetization at equilibrium is along z

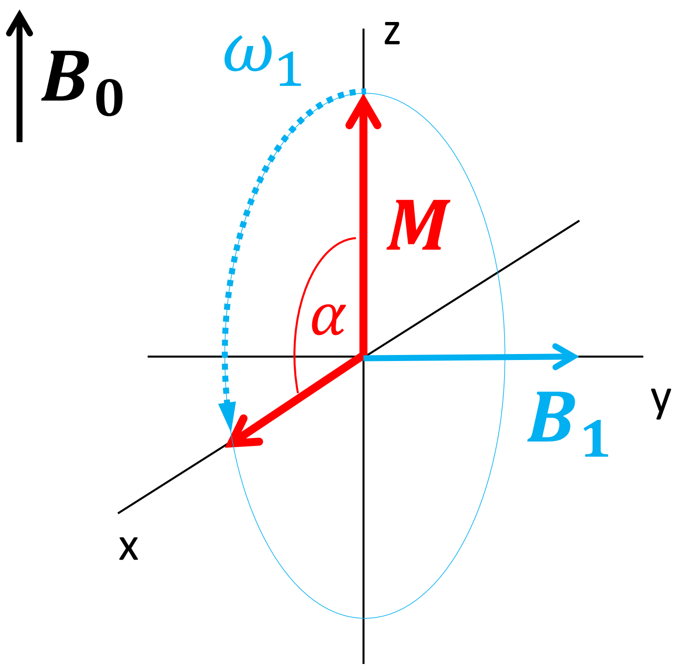


Magnetization in the x-y plane precesses with frequency $\omega = -\gamma B$ (Larmor frequency)



Magnetization is rotated from z axis to the x-y plane (or vice versa) by a pulse of perpendicular (horizontal) magnetic field $\boldsymbol{B_1}$ that oscillates with angular velocity $\boldsymbol{\omega_{rf}} \approx \boldsymbol{\omega}$ (Larmor frequency)

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$)



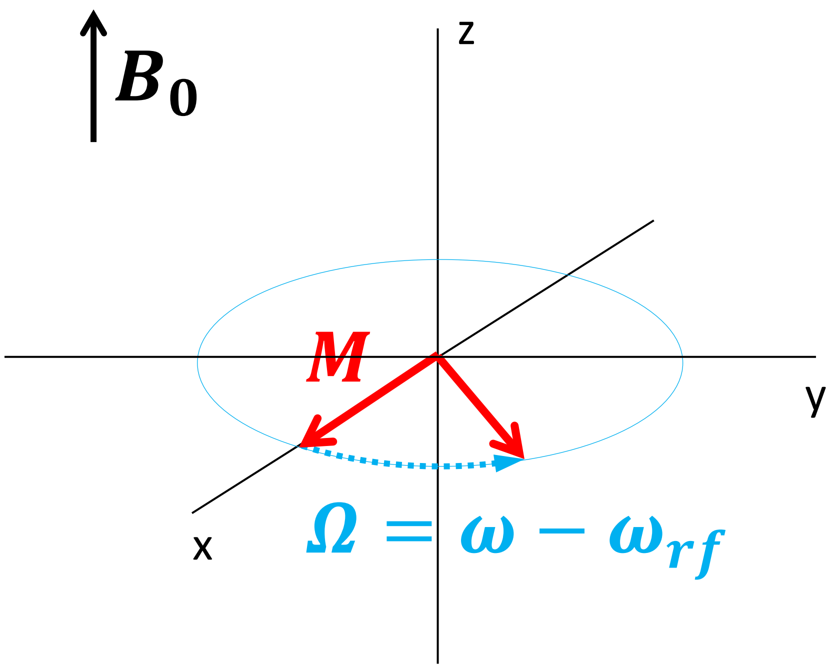
90° pulse:

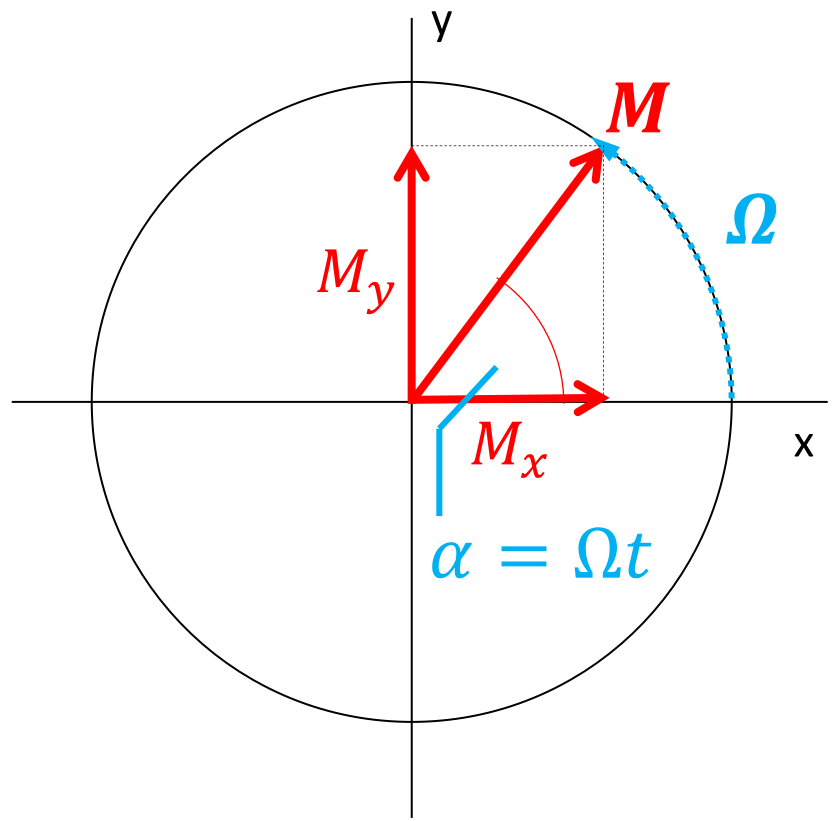
Turn on $\boldsymbol{B_1}$ for time t such that

$\alpha = \omega_1 t = |-\gamma B_1| t = \frac{\pi}{2}$

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$)

Magnetization in the x-y plane precesses with frequency $\boldsymbol{\Omega}$



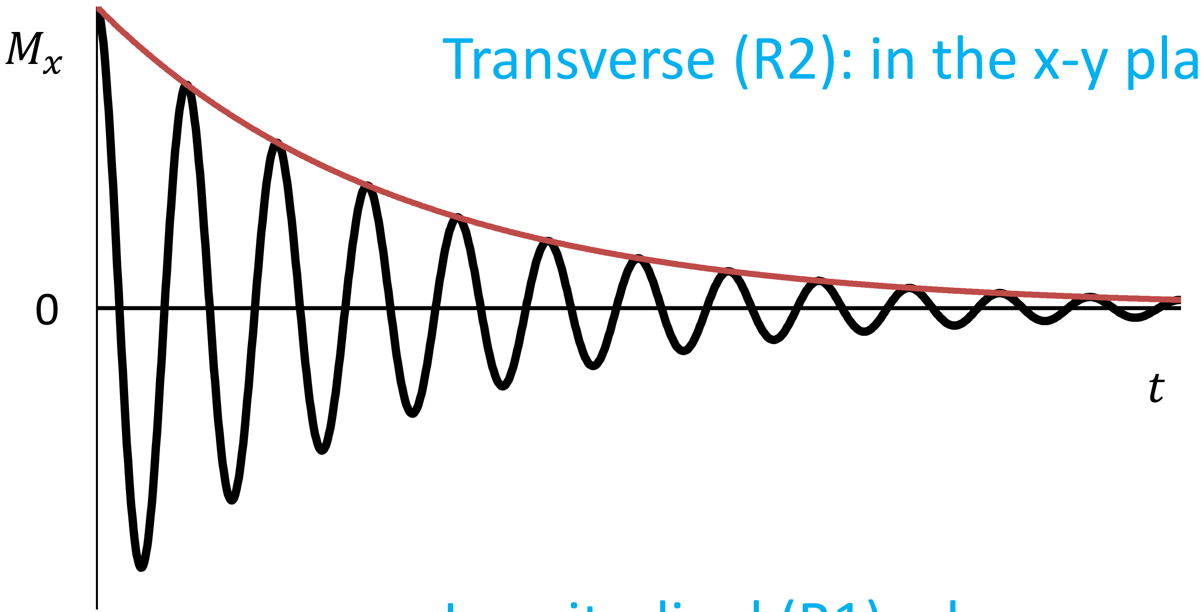


$$M_x = M_0 \cos \Omega t$$
$$M_y = M_0 \sin \Omega t$$

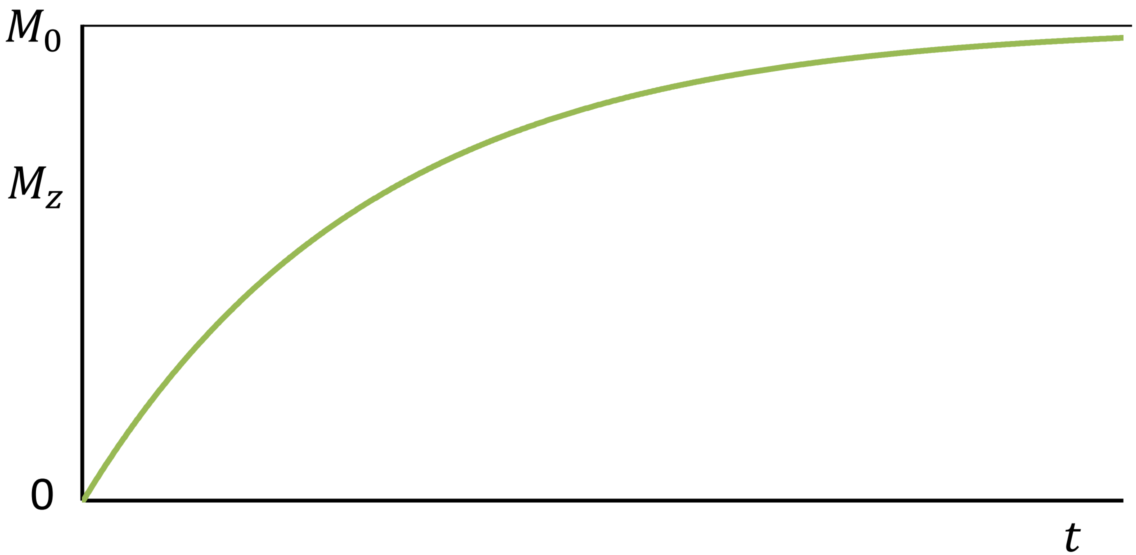
$$M_{xy} = M_x + iM_y = M_0 e^{i\omega t}$$

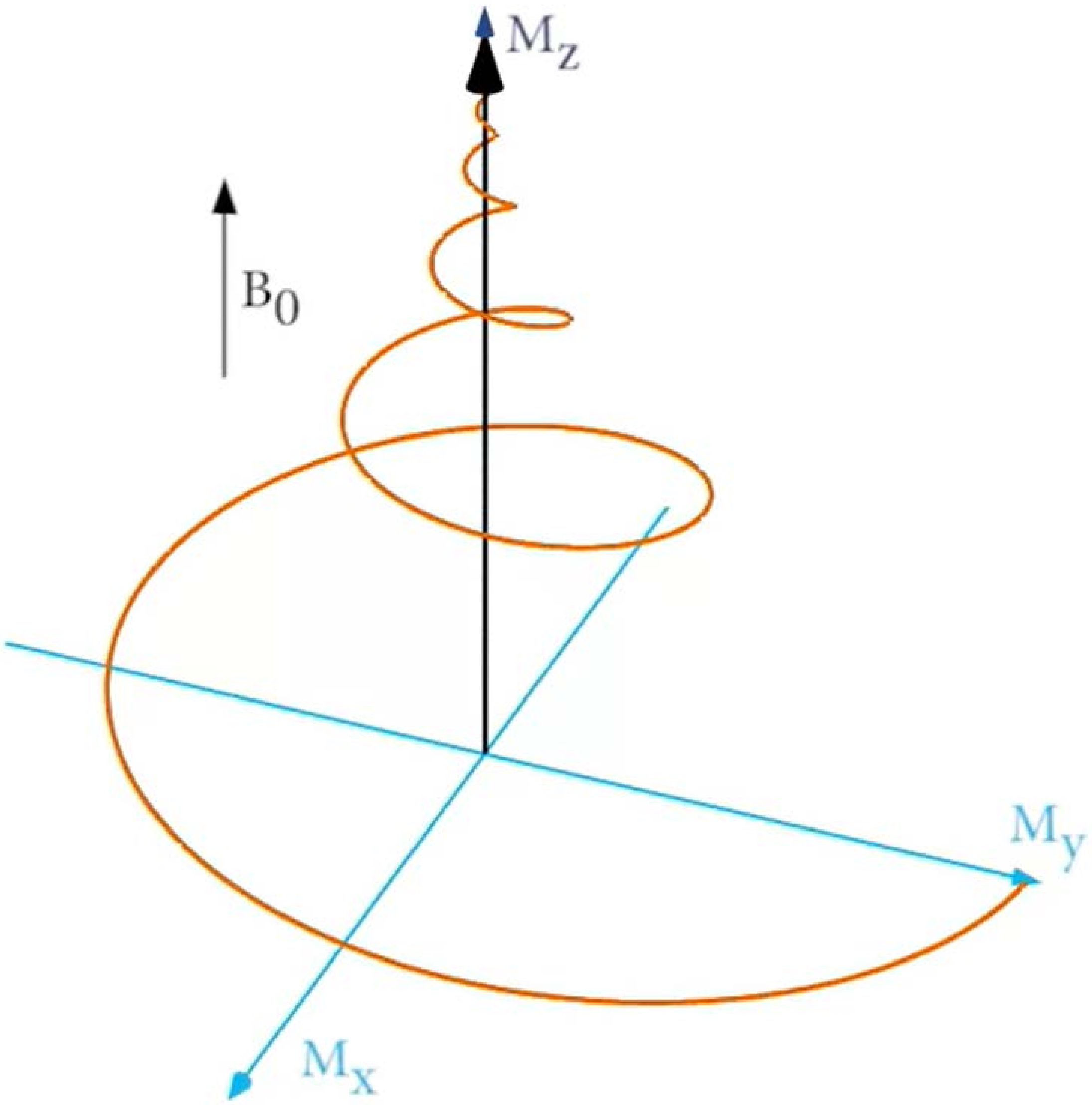
Relaxation

Transverse (R2): in the x-y plane



Longitudinal (R1): along z



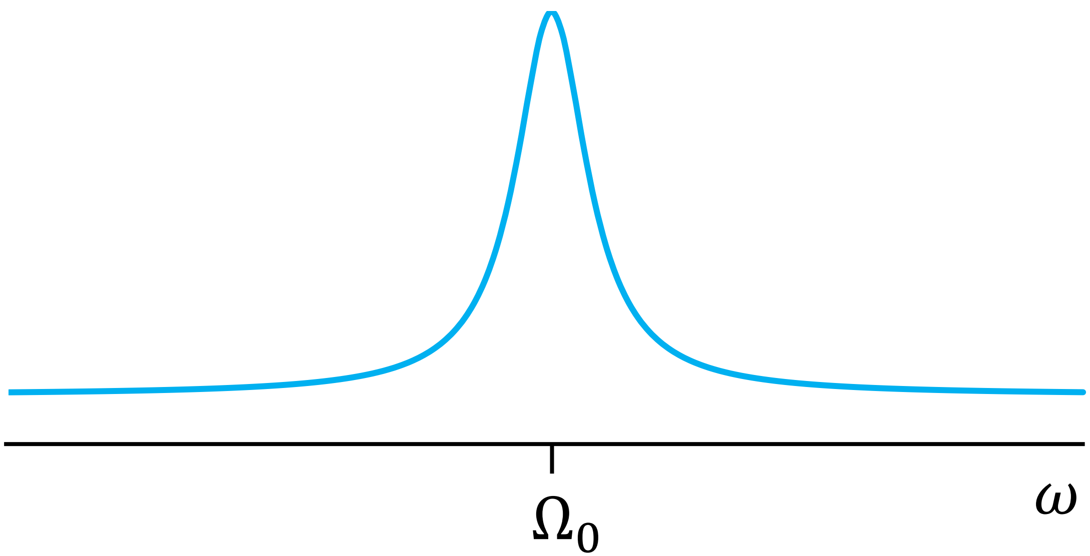


https://en.wikipedia.org/wiki/Relaxation_(NMR)

Oscillation $s(t) = M_0 e^{-R_2 t} e^{i\Omega_0 t}$

Fourier transform

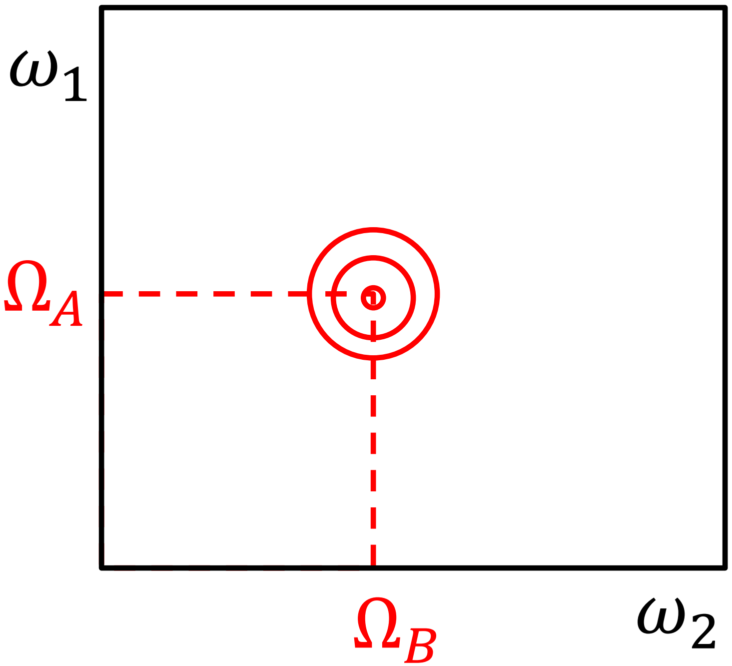
$$S(\omega) = \int_{-\infty}^{\infty} s(t) e^{-i\omega t}\, dt$$



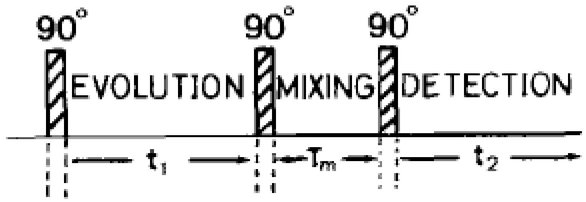
2-dimensional (2D) NMR spectroscopy

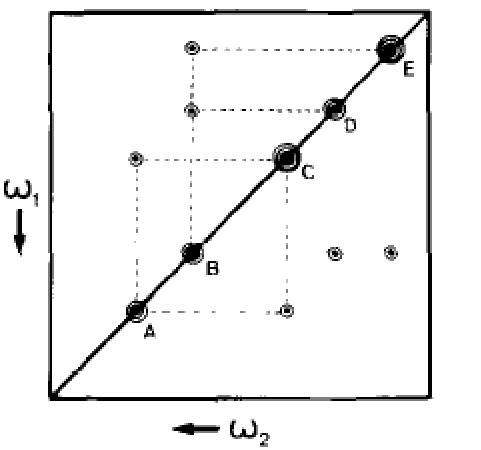
Correlation between nuclear dipoles

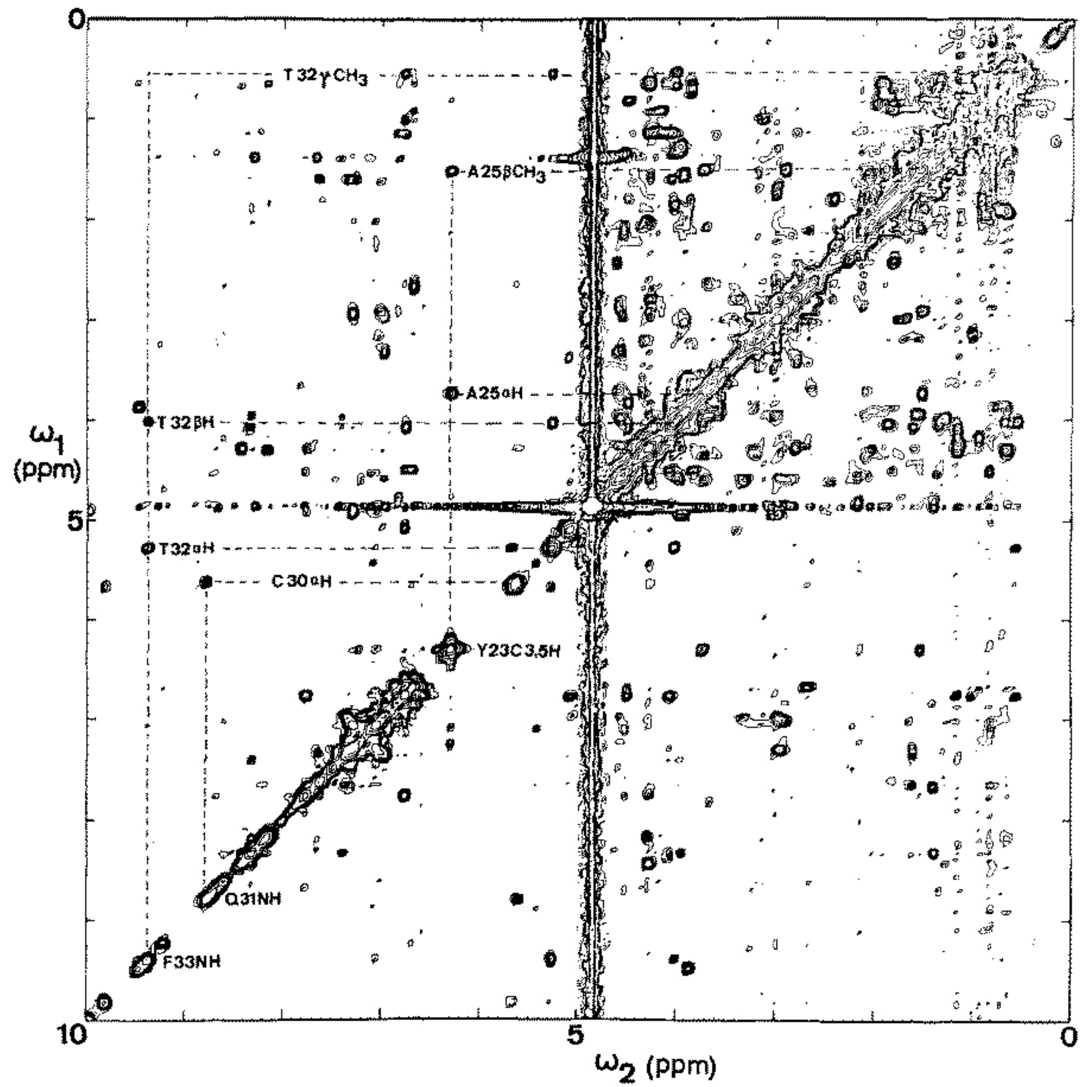
- Through-bond interactions (J-coupling) < 4 bonds apart
- Through-space interactions (nuclear Overhauser effect, NOE) < 5 Å apart



The first 2D spectrum of a protein (BPTI)

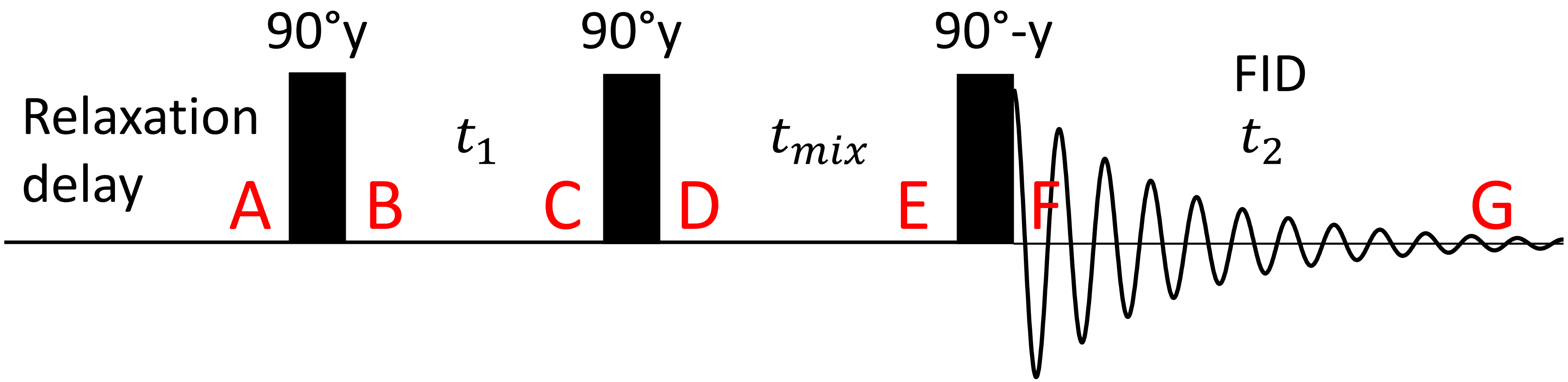


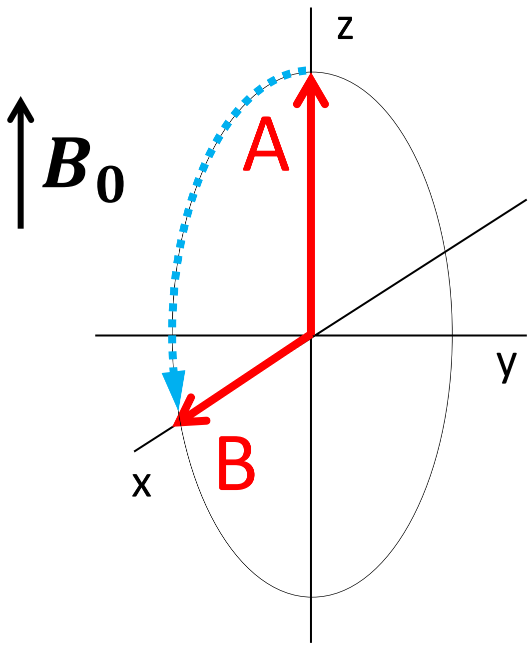




Kumar A, Ernst RR, Wüthrich K. A two-dimensional nuclear Overhauser enhancement (2D NOE) experiment for the elucidation of complete proton-proton cross-relaxation networks in biological macromolecules. Biochem Biophys Res Commun. 1980 Jul 16;95(1):1-6.

2D NMR spectroscopy

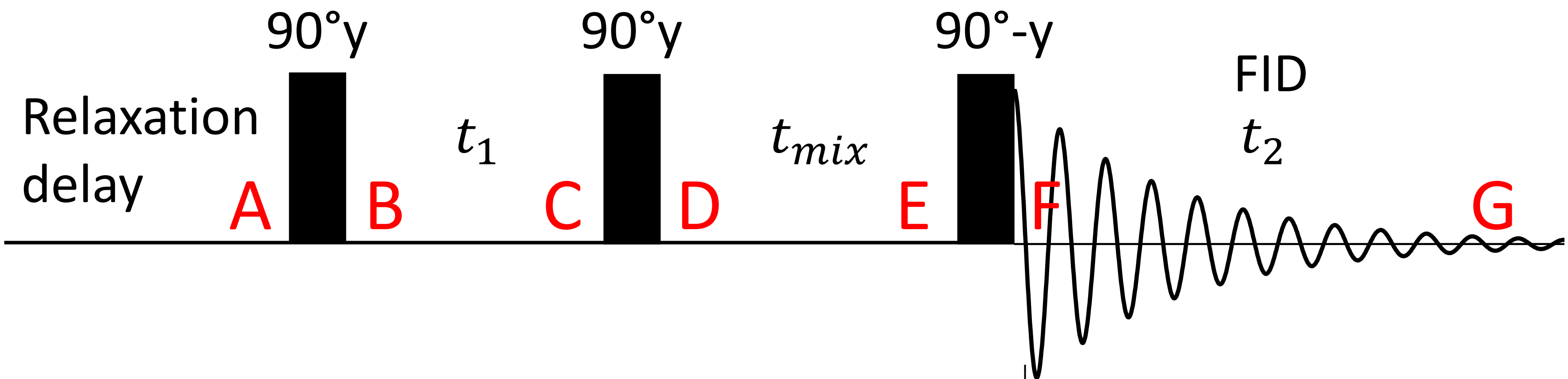


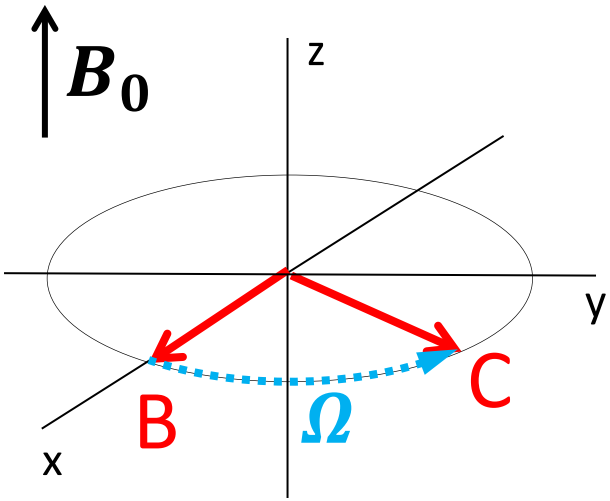


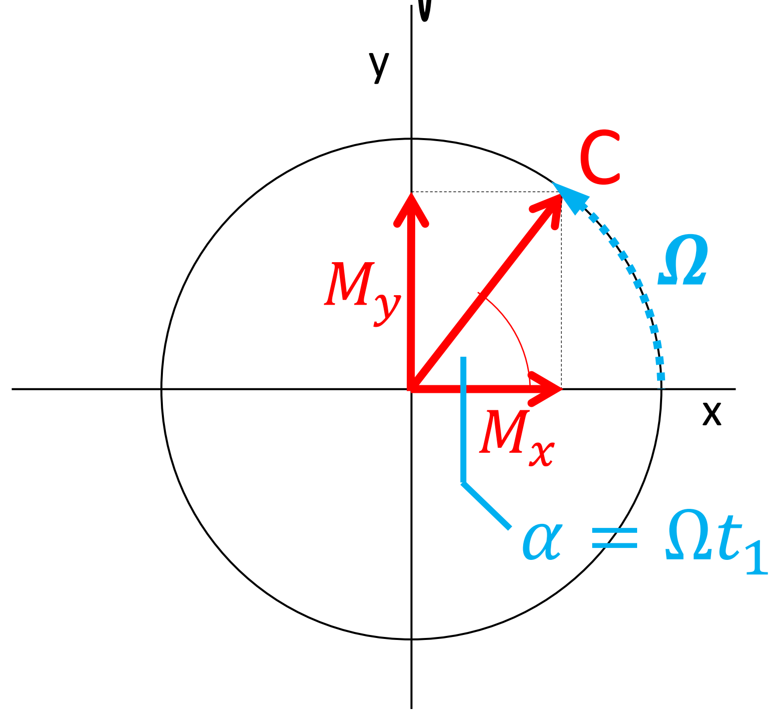
A: $M_z = M_0$

B: $M_x = M_0$

2D NMR spectroscopy



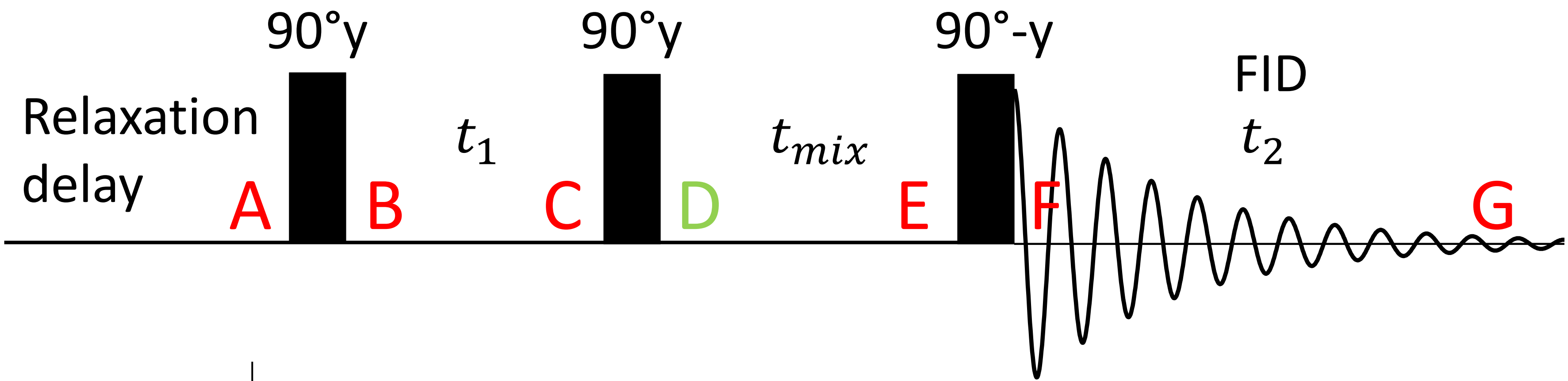


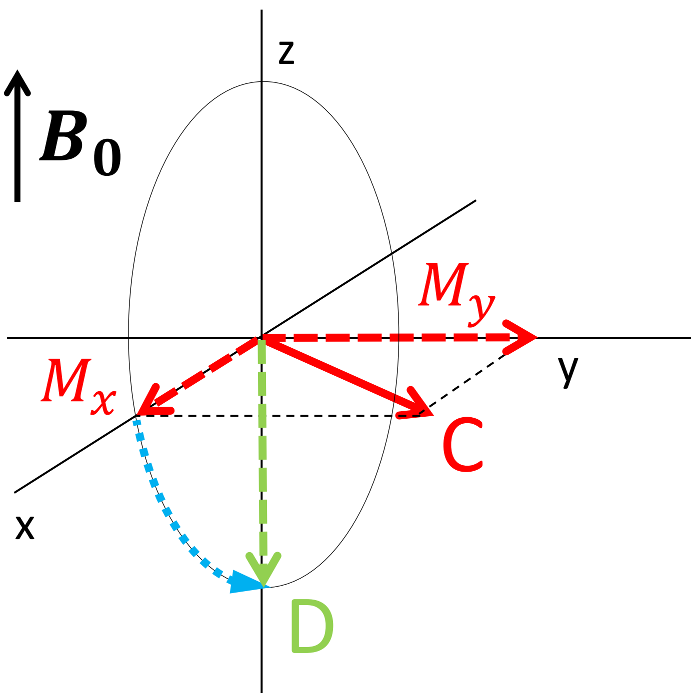


C: $M_x = M_0 \cos \Omega t_1$

$M_y = M_0 \sin \Omega t_1$

2D NMR spectroscopy





D: $M_z = -M_0 \cos \Omega t_1$

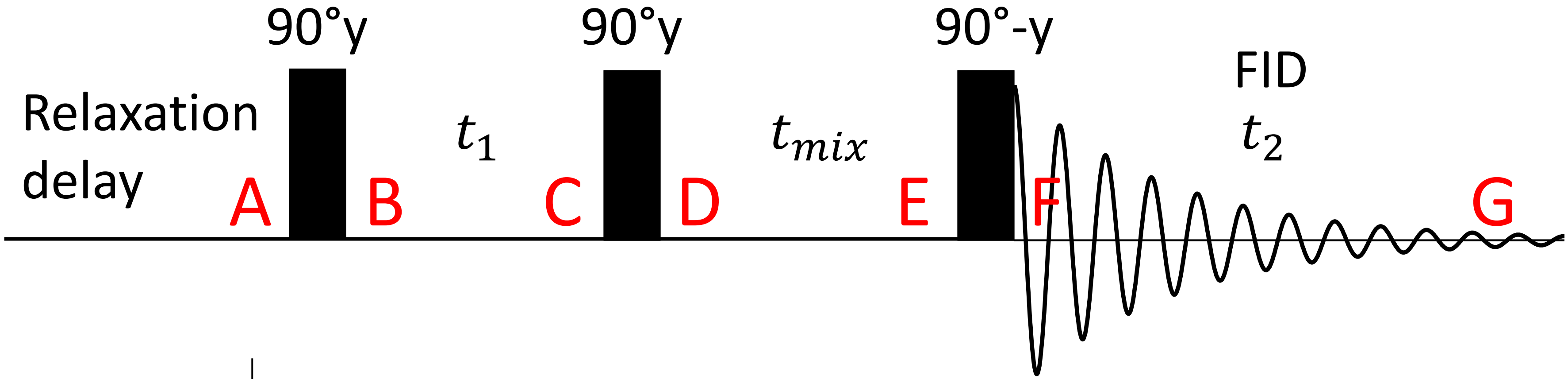
$M_y = M_0 \sin \Omega t_1$

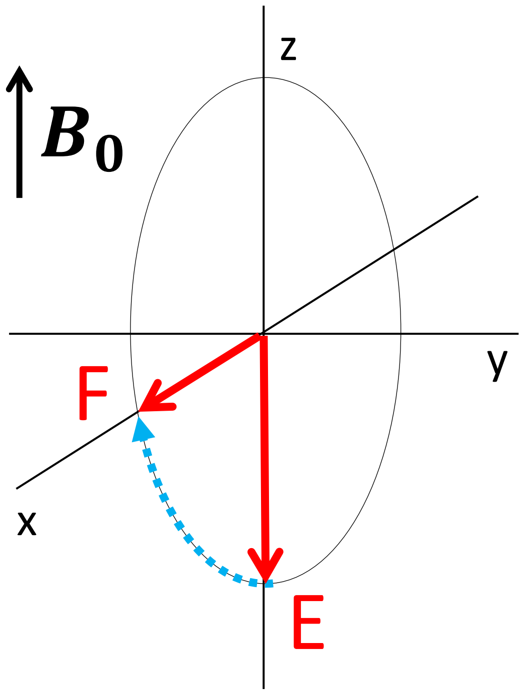
(ignore M_y, it will get cancelled by phase cycling and/or gradients

E: $M_z = -M_0 \, a \cos \Omega t_1$

Relaxation during t_{mix}

2D NMR spectroscopy





E: $M_z = -M_0 \text{ a} \cos \Omega t_1$

F: $M_x = M_0 \text{ a} \cos \Omega t_1$

G: $M_x = M_0 \text{ a} \cos \Omega t_1 e^{-R_2 t_2} \cos \Omega t_2$

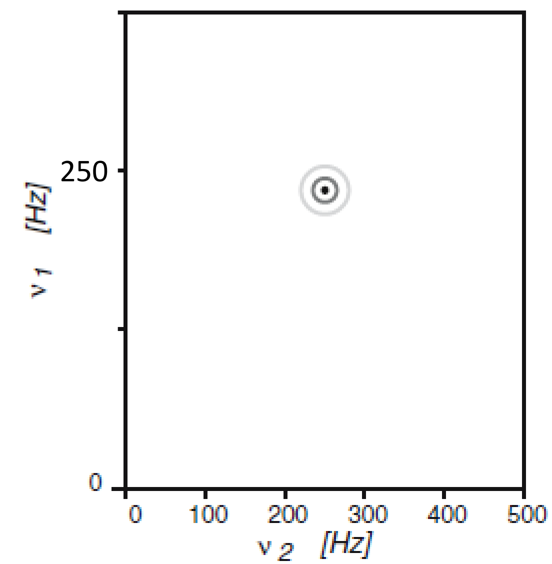
$M_y = M_0 \text{ a} \cos \Omega t_1 e^{-R_2 t_2} \sin \Omega t_2$

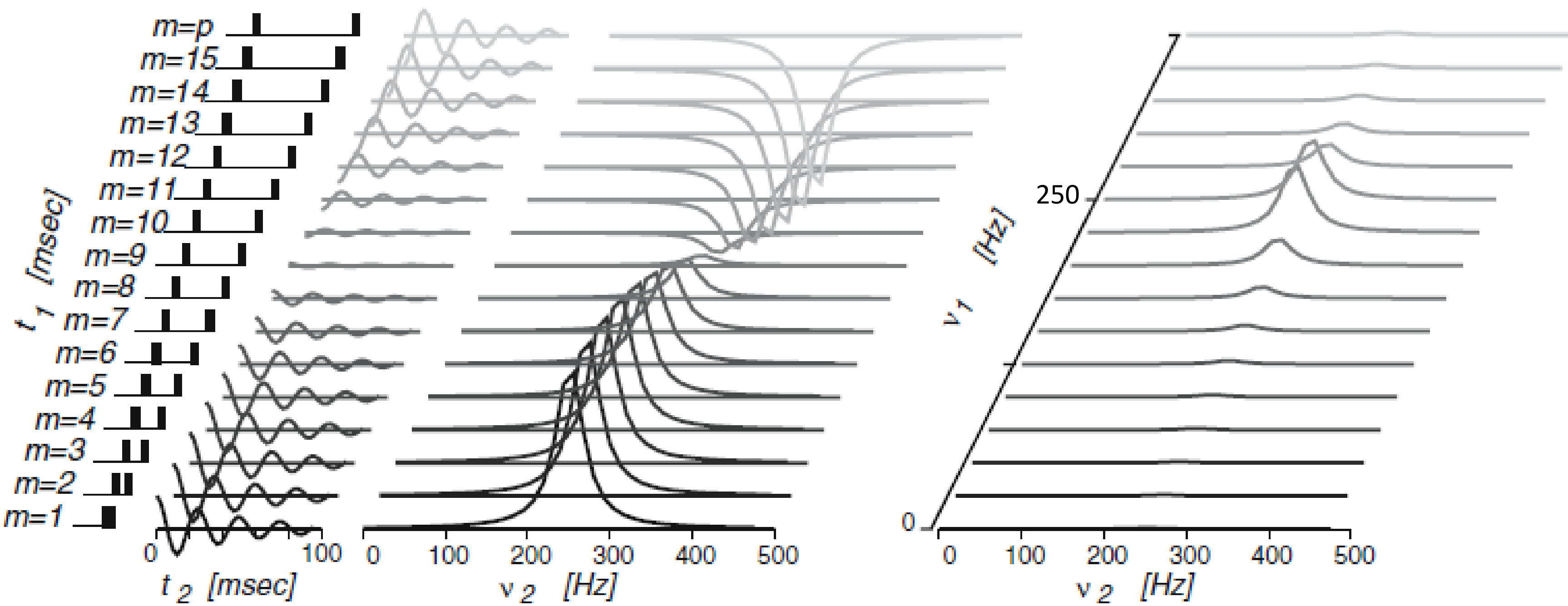
$M_{xy} = M_0 \text{ a} \cos \Omega t_1 e^{-R_2 t_2} e^{i \Omega t_2}$

$$M_{xy} = M_0\, a \boxed{\cos \Omega t_1}\, e^{-R_2 t_2} e^{i\Omega t_2}$$

Amplitude modulation

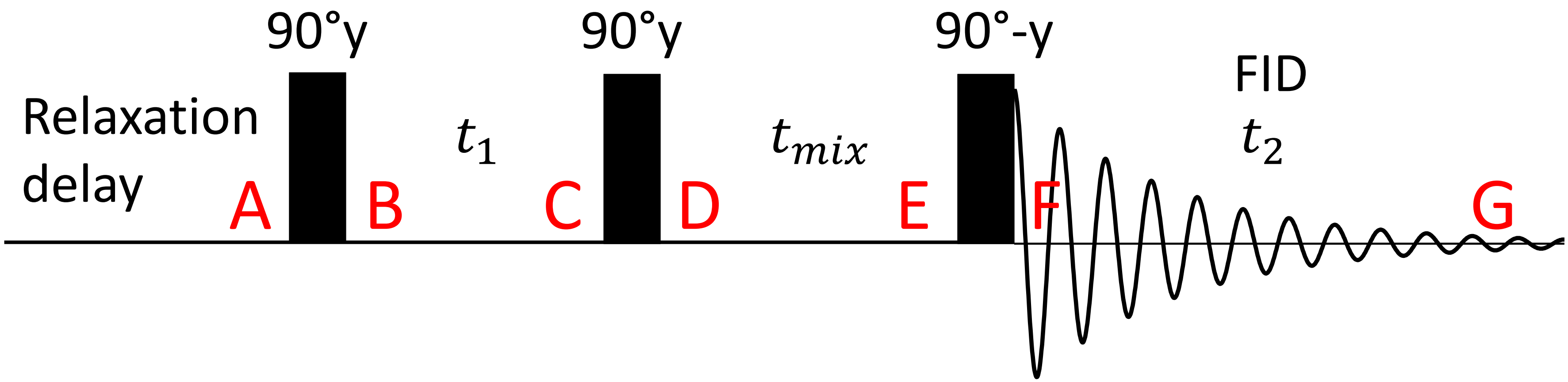
- Acquire data with increasing t_1
- Fourier transform each FID (in t_2)
- Fourier transform the spectra in t_1





Rule, G.S. and Hitchens, T.K. Fundamentals of Protein NMR Spectroscopy, 2006, Springer

2-dimensional (2D) NMR spectroscopy



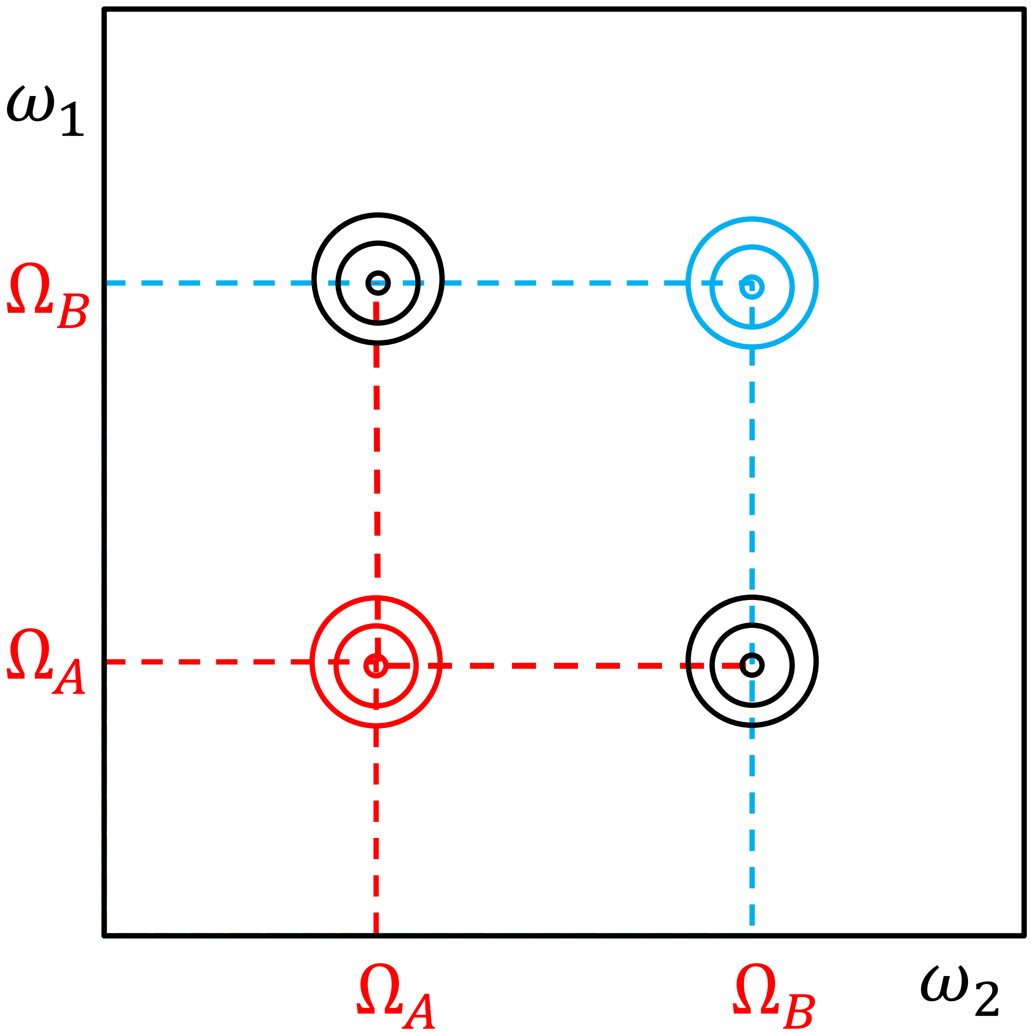
Nucleus A | **Nucleus B**

D: $M_z = -M_A \cos \Omega_A t_1$ | $M_z = -M_B \cos \Omega_B t_1$

E: $-M_A\, a_{AA} \cos \Omega_A t_1$ | $-M_B\, a_{BB} \cos \Omega_B t_1$

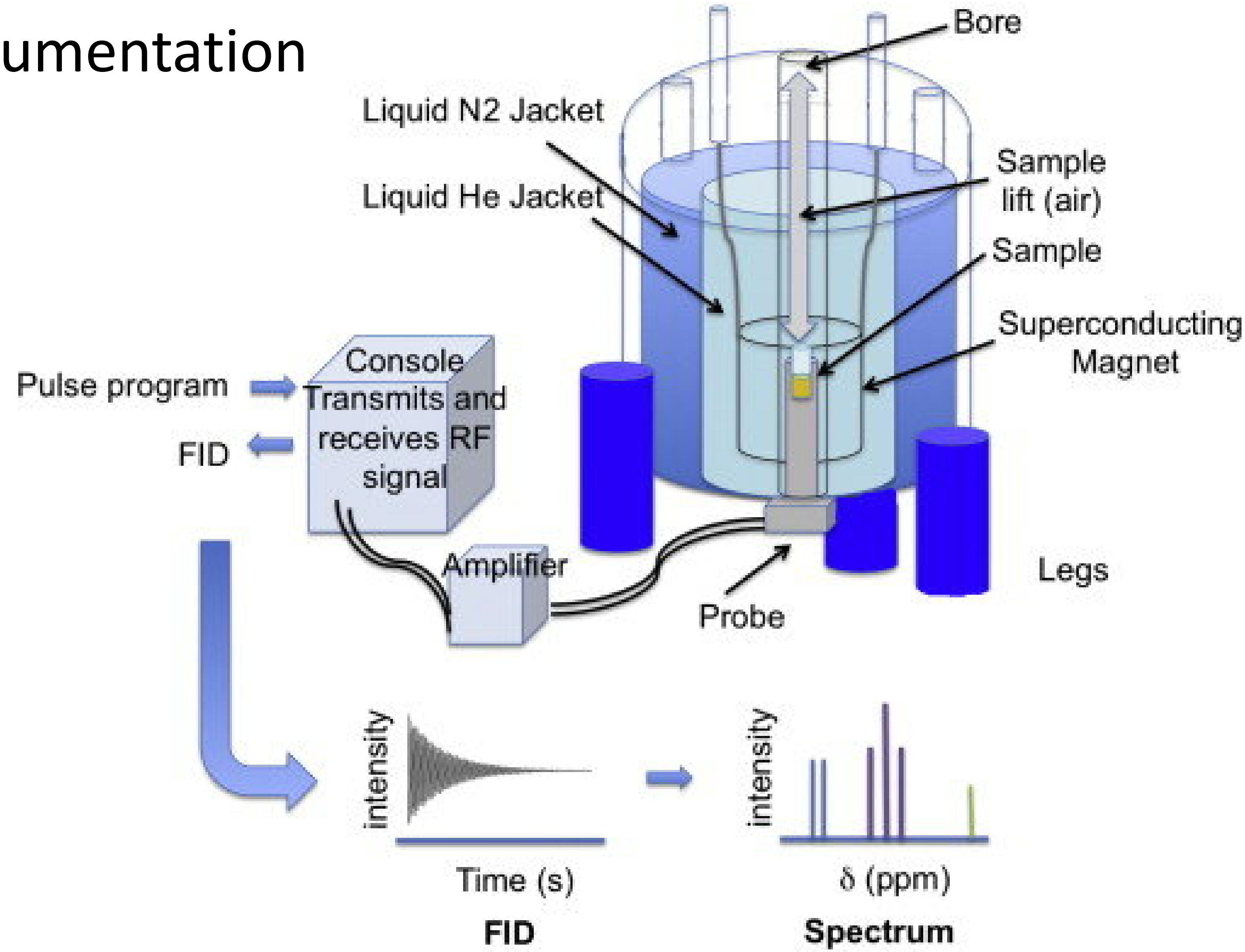
$-M_B\, a_{BA} \cos \Omega_B t_1$ | $-M_A\, a_{AB} \cos \Omega_A t_1$

G: $(\ )e^{-R_2 t_2} e^{i\Omega_A t_2}$ | $(\ )e^{-R_2 t_2} e^{i\Omega_B t_2}$



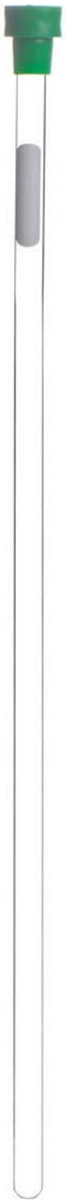
ω_1
Ω_B
Ω_A
Ω_A
Ω_B
ω_2

Instrumentation

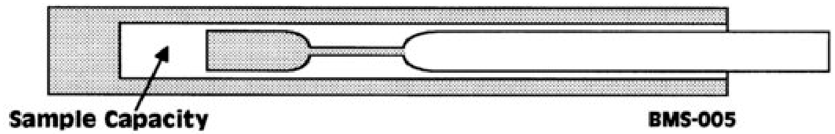


Rankin, Naomi & Preiss, David & Welsh, Paul & Burgess, Karl & Nelson, Scott & Lawlor, Debbie & Sattar, Naveed. (2014). The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective. Atherosclerosis. 237. 287–300.


VARIAN



- Typical NMR tube diameter: 5 mm
- Sample volume: > 600 µL
- Smaller volume (200-300 µL) in Shigemi tubes



- Concentration for structure determination: several hundred µM
- Lower concentration for ligand binding
- Signal to noise ratio (S/N) $\alpha \sqrt{n}$
- Cryoprobe: electronics cooled to ~ 20K, higher S/N (up to 4 fold), lower concentrations possible

Setting up an NMR experiment

- Tuning: optimize efficiency of energy transfer between the coil and the sample
- ^{2}H Lock: long-term stability of the magnetic field
- Shimming: optimize homogeneity of the magnetic field within the sample
- Calibration of pulses: experimentally determine the duration of a 90° pulse for a given power level
- Data acquisition